

Claims:

1. A nucleic acid molecule which provides one or more accessory functions for supporting recombinant AAV (rAAV) virion production in a suitable host cell, said molecule comprising:
 - (a) an adenovirus VA RNA coding region;
 - (b) an adenovirus E4 ORF6 coding region;
 - (c) an adenovirus E2A 72 kD coding region;
 - (d) an adenovirus E1A coding region; and
 - (e) an adenovirus E1B region lacking an intact E1B55k coding region.
2. An accessory function vector comprising the nucleic acid molecule of claim 1.
3. The accessory function vector of claim 2, wherein said vector is a plasmid.
4. The accessory function vector of claim 3, further comprising at least one heterologous promoter region operably linked to one or more of said coding regions.
5. The accessory function vector of claim 3 wherein an inducible promoter is operably linked to the E2A 72 kD coding region.
6. The accessory function vector of claim 5 wherein the inducible promoter is a small molecule-regulated promoter.
7. The accessory function vector of claim 6 wherein the promoter is an ecdysone-inducible promoter.
8. The accessory function vector of claim 3 wherein an inducible promoter is operably linked to the E1A coding region.

9. The accessory function vector of claim 8 wherein the inducible promoter is a small molecule-regulated promoter.
10. The accessory function vector of claim 9 wherein the promoter is an ecdysone-inducible promoter.
11. The nucleic acid molecule of claim 1, wherein said nucleic acid molecule lacks adenoviral early gene regions E2B and E3.
12. The nucleic acid molecule of claim 1, wherein said nucleic acid molecule provides accessory functions capable of supporting efficient rAAV virion production in an human 293 host cell.
13. The nucleic acid molecule of claim 12, wherein one or more of (a) - (e) are derived from an adenovirus type-2 or type-5 genome.
14. The nucleic acid molecule of claim 1, wherein the nucleic acid molecule provides accessory functions capable of supporting efficient recombinant AAV (rAAV) virion production in a suitable host cell that is not infectable by adenovirus or is not capable of supporting adenovirus replication.
15. The nucleic acid molecule of claim 14, wherein one or more of (a) - (e) are derived from an adenovirus type-2 or type-5 genome.
16. An accessory function vector comprising the nucleic acid molecule of claim 11.
17. The accessory function vector of claim 16, wherein said vector is a plasmid.
18. An accessory function vector system, comprising:
(a) a nucleic acid sequence that provides adenovirus VA RNAs;

- (b) an adenovirus E4 ORF6 coding region;
- (c) an adenovirus E2A 72 kD coding region;
- (d) an adenovirus E1A coding region; and
- (e) an adenovirus E1B region lacking an intact E1B55k coding region;

5 wherein (a)-(e) are included on more than one accessory function vector of said system.

19. The accessory function vector system of claim 18, wherein said vectors are plasmids.

10 20. A host cell comprising the accessory function vector of claim 2.

21. A host cell comprising the accessory function vector of claim 16.

22. A host cell comprising the accessory function vector system of claim 18.

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23. A cell capable of producing recombinant AAV (rAAV) virions when transfected with an AAV vector, said cell comprising the host cell of claim 20, wherein the host cell further comprises an AAV helper construct that is capable of being expressed in said cell to provide AAV helper functions.

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24. A method of producing recombinant AAV (rAAV) virions, comprising:

- (a) introducing an AAV vector into a suitable host cell;
- (b) introducing an AAV helper construct into the host cell, said helper construct comprising AAV coding regions that are expressed in the host cell to complement AAV helper functions missing from said AAV vector;
- (c) introducing the accessory function vector of claim 2 into the host cell, said accessory function vector providing accessory functions for supporting efficient rAAV virion production in the host cell; and
- (d) culturing the host cell to produce rAAV virions.

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25. A method of producing recombinant AAV (rAAV) virions, comprising:
- (a) introducing an AAV vector into a suitable host cell;
 - (b) introducing an AAV helper construct into the host cell, said helper construct comprising AAV coding regions that are expressed in the host cell to complement AAV helper functions missing from said AAV vector;
 - (c) introducing the accessory function vector of claim 16 into the host cell, said accessory function vector providing accessory functions for supporting efficient rAAV virion production in the host cell; and
 - (d) culturing the host cell to produce rAAV virions.

26. A method of producing recombinant AAV (rAAV) virions, comprising:
- (a) introducing an AAV vector into a suitable host cell;
 - (b) introducing an AAV helper construct into the host cell, said helper construct comprising AAV coding regions that are expressed in the host cell to complement AAV helper functions missing from said AAV vector;
 - (c) introducing the accessory function vector system of claim 18 into the host cell, said accessory function vector system providing accessory functions for supporting efficient rAAV virion production in the host cell; and
 - (d) culturing the host cell to produce rAAV virions.

27. A method of producing recombinant AAV (rAAV) virions comprising the steps of:
- (a) introducing an AAV *rep* coding region into a suitable host cell;
 - (b) introducing AAV vector sequences into the host cell;
 - (c) infecting the host cell with a recombinant helper virus, wherein the recombinant helper virus comprises accessory functions and an AAV *cap* coding region; and
 - (d) culturing the host cell to produce rAAV virions;

wherein steps (a) and (b) may be performed in any order.

28. The method of claim 27, wherein the AAV vector sequences are introduced by infection.
29. The method of claim 27, wherein the AAV vector sequences are episomal.
- 5 30. The method of claim 27, wherein the AAV vector sequences are integrated.
31. The method of claim 27, wherein the recombinant helper virus is a recombinant adenovirus.
- 10 32. The method of claim 31, wherein the *cap* coding region replaces the adenoviral E3 region.
33. The method of claim 27, wherein the *cap* coding region is operably linked to a
- 15 heterologous promoter.
34. The method of claim 33, wherein the *cap* coding region is operably linked to an adenovirus major late promoter.
- 20 35. The method of claim 33, wherein the *cap* coding region is operably linked to an inducible promoter.
36. The method of claim 35, wherein the *cap* coding region is operably linked to an ecdysone-inducible promoter.
- 25 37. A method of producing recombinant AAV (rAAV) virions comprising the steps of:
- (a) introducing an AAV helper construct into a suitable host cell, said AAV helper construct comprising AAV coding regions that are expressed in the host
- 30 cell to complement rAAV virion production in the host cell;

- 5 (b) introducing an accessory function system into the host cell, said accessory function system providing accessory functions for supporting rAAV virion production in the host cell;
- (c) introducing an AAV vector by infection of the host cell; and
- (d) culturing the host cell to produce rAAV virions.

38. The method of claim 37, wherein the AAV vector is introduced into the host cell by infection with a recombinant AAV virion.

- 10 39. The method of claim 37, wherein the accessory function system comprises an adenovirus VA RNA coding region, an adenovirus E4 ORF6 coding region, an adenovirus E2A 72kD coding region, an adenovirus E1A coding region, and an adenovirus E1B coding region.

- 15 40. The method of claim 39, wherein the E1B coding region lacks an intact E1B55k coding region.

41. A system for the production of recombinant AAV (rAAV) comprising:

- 20 (a) a first nucleic acid comprising an SV40 large T-antigen coding region that is operably linked to an inducible promoter;
- (b) a second nucleic acid comprising an adenovirus E1A coding region;
- (c) a third nucleic acid comprising an adenovirus E1B coding region;
- (d) a fourth nucleic acid comprising an Epstein-Barr virus nuclear antigen 1 coding region;
- 25 (e) a fifth nucleic acid comprising an adenovirus VA RNA coding region;
- (f) a sixth nucleic acid comprising an adenovirus E4 ORF6 coding region;
- (g) a seventh nucleic acid comprising AAV vector sequences; and
- (h) an eighth nucleic acid comprising an AAV *rep* and *cap* coding region, an adenovirus E2A gene, an SV40 origin of replication, an Epstein-Barr virus latent

origin of replication, and a selectable marker, wherein said eighth nucleic acid lacks an intact AAV p5 promoter region.

42. A host cell comprising the system of claim 41.

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43. A system for the production of recombinant AAV (rAAV) comprising:

- 10 (a) a first nucleic acid comprising an SV40 large T-antigen coding region that is operably linked to an inducible promoter, an adenovirus E1A coding region, an adenovirus E1B coding region, an Epstein-Barr virus nuclear antigen 1 coding region, an adenovirus VA RNA coding region, an adenovirus E4 ORF6 coding region, and a selectable marker;
- (b) a second nucleic acid comprising AAV vector sequences and a selectable marker; and
- 15 (c) a third nucleic acid comprising AAV *rep* and *cap* coding regions, an adenovirus E2A gene, an SV40 origin of replication, an Epstein-Barr virus latent origin of replication, and a selectable marker, wherein said third nucleic acid lacks an intact AAV p5 promoter region.

44. A host cell comprising the system of claim 43.

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45. The system of claim 43, wherein the SV40 large T-antigen coding region is mutated to eliminate transforming activity.

46. The system of claim 43, wherein the E1A coding region is operably linked to an inducible promoter.

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47. The system of claim 43, wherein the E4 ORF6 coding region is operably linked to an adenovirus E4 promoter.

48. The system of claim 43, wherein the SV40 large T-antigen coding region is operably linked to an ecdysone-inducible promoter, the E1A coding region is operably linked to an ecdysone-inducible promoter, and the second nucleic acid further comprises ecdysone receptor subunit coding regions.

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49. The system of claim 48, wherein the E2A coding region is operably linked to an ecdysone-inducible promoter.

50. A method of producing recombinant AAV (rAAV) comprising the steps of:

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(a) introducing a first nucleic acid comprising an adenovirus VA RNA coding region and an E4 ORF6 coding region into a host cell, wherein the host cell comprises an adenovirus E1A coding region and an adenovirus E1B coding region;

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(b) introducing a second nucleic acid comprising AAV vector sequences into the host cell; and

(c) introducing a third nucleic acid comprising AAV *rep* and *cap* coding regions and an adenovirus E2A coding region into the host cell such that the third nucleic acid is maintained as an episome in the host cell;

wherein step (a) - (c) may be performed in any order.

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51. The method of claim 50, further comprising the step of introducing a fourth nucleic acid comprising a viral nuclear antigen coding region into the host cell and wherein the third nucleic acid further comprises a viral origin of replication, such that the viral nuclear antigen and viral origin of replication function to maintain the third nucleic acid as an episome in the host cell.

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52. The method of claim 50, further comprising the step of introducing a fourth nucleic acid comprising an SV40 large T-antigen coding region into the host cell and wherein the third nucleic acid further comprises an SV40 origin of replication.

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53. The method of claim 52, further comprising the step of introducing a fifth nucleic acid comprising an Epstein-Barr virus nuclear antigen 1 coding region into the host cell and wherein the third nucleic acid further comprises an Epstein-Barr virus latent origin of replication.

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54. The method of claim 52, wherein the SV40 large T-antigen coding region is operably linked to an inducible promoter.

55. The method of claim 54, wherein the SV40 large T-antigen coding region is
10 operably linked to an ecdysone-inducible promoter.

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